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(54) Title: DCR5, A BMP-BINDING PROTEIN, AND APPLICATIONS THEREOF		
(57) Abstract <p>DCR5, a protein related to DAN (Differential-screening-selected gene Aberrative in Neuroblastoma) and related nucleic acids are provided. Included are natural DCR5 homologs from several species and proteins comprising a DCR5 domain having specific activity, particularly the ability to antagonize a bone morphogenetic protein. The proteins may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are isolated hybridization probes and primers capable of specifically hybridizing with the disclosed genes, specific binding agents and methods of making and using the subject compositions.</p>		

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DCR5, A BMP-BINDING PROTEIN, AND APPLICATIONS THEREOF

This application claims priority of U.S. Serial No. 60/097,296, filed August 20, 1998, the contents of which is incorporated by reference into this application.

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Field of the Invention

The field of this invention is proteins which regulate cell function, and in particular, antagonize bone morphogenetic proteins.

10

Background

Natural regulators of cellular growth, differentiation and function have provided important pharmaceuticals, clinical and laboratory tools, and targets for therapeutic intervention. A variety of such regulators have been shown to have profound effects on basic cellular differentiation and developmental pathways. For example, the recently cloned cerberus protein induces the formation of head structures in anterior endoderm of vertebrate embryos. Similarly, the Noggin protein induces head structures in vertebrate embryos, and can redirect mesodermal fates from ventral fates, such as blood and mesenchyme, to dorsal fates such as muscle and notochord and can redirect epidermal fates to anterior neural fates. The activities of chordin are similar to those of Noggin, reflecting a common mechanism of action - namely antagonizing bone morphogenetic proteins (BMP) and thereby preventing their function. BMPs have diverse biological activities in different biological contexts, including the induction of cartilage, bone and connective tissue, and roles in kidney, tooth, gut, skin and hair development.

Different members of the TGF β superfamily can instruct cells to follow different fates, for example TGF β induces neural crest to form smooth muscle, while BMP2 induces the same cells to become neurons. In *Xenopus* experiments, dissociated animal cap cells (prospective ectoderm) become epidermis in response to BMP4 but become mesoderm in response to activin.

Since the sequence identity between activin and BMP4 is low, it is not surprising that they induce different fates. It is more surprising that members of the BMP subfamily, which are quite closely related in sequence, can induce distinct fates. A striking example results from implantation of a matrix
5 impregnated with a BMP into muscle; when the effects are monitored histologically, BMP2, 4 and 7 induce endochondral bone formation, whereas a related molecule BMP12/GDF7 induces connective tissue similar to tendon. Similarly, BMP4 can induce cell death in the hindbrain neural crest, while the related protein dorsalin does not.

10 Since different BMP family members can induce different fates, then BMP antagonists that have specificity in blocking subsets of BMPs could change the balance of BMPs that are presented to a cell, thus altering cell fate. In view of the importance of relative BMP expression in human health and disease, regulators of cellular function and BMP function in particular, such as Noggin and cerberus,
15 provide valuable reagents with a host of clinical and biotechnological applications. The present invention relates to a new family of regulators of cellular function.

Relevant Literature

20 Bouwmeester, et al. (1996) Nature 382: 595-601 describe the cloning of Xenopus cerberus gene; Lamb, T. M., et al. (1993) Science 262: 713-718; Smith, W. C., et al. (1992) Cell 70: 829-840; Smith, W. C., et al. (1993) Nature 361: 547-549; and Zimmerman, L. B., et al. (1996) Cell 86: 599-606 describe the isolation and function
25 of the Noggin protein. Piccolo, S., et al. (1996) Cell 86: 589-598; Sasai, Y., et al. (1995) Nature 376: 333-336; and Sasai, Y., et al. (1994) Cell 79: 779-790 relate to the chordin protein. Enomoto et al. (1994) Oncogene 9: 2785-2791 and Ozaki, et al. (1996) Jpn. J. Cancer Res. 87: 58-61 describe human and murine homologs of the DAN gene. Hsu, et al. (1998) Mol Cell 1:673-683 describing Gremlin from a
30 variety of species, including human; Minabe-Saegusa, C., et al. (1998) Dev Growth Differ 40:343-353 which describes mouse PRDC.

SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to DCR5, a protein related to Gremlin, DAN (Differential-screening-selected gene A aberrant in Neuroblastoma) and Cerberus, and related nucleic acids. Included are natural DCR5 homologs from different species, as well as proteins comprising a DCR5 domain and having DCR5-specific activity, particularly the ability to antagonize a bone morphogenetic protein. The proteins may be produced recombinantly from transformed host cells with the subject nucleic acids. The invention provides isolated hybridization probes and primers capable of specifically hybridizing with the disclosed genes, specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g., genetic hybridization screens for DCR5 transcripts), therapy (e.g., gene therapy to modulate DCR5 gene expression) and in the biopharmaceutical industry (e.g., reagents for screening chemical libraries for lead pharmacological agents).

Preferred applications of the subject DCR5 proteins include modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous DCR5 protein under conditions whereby the added protein specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. Also preferred are methods for screening for biologically active agents, which methods involve incubating a DCR5 protein in the presence of an extracellular DCR5 protein-specific binding target and a candidate agent, under conditions whereby, but for the presence of the agent, the protein specifically binds the binding target at a reference affinity; detecting the binding affinity of the protein to the binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that the agent modulates the binding of the protein to the binding target.

Another preferred embodiment of the invention is a method of treatment of a human or animal body by administering a therapeutic dosage of a human DCR5 polypeptide as wherein the treatment is regulation of cartilage and bone growth.

An additional preferred embodiment of the invention is a ligandbody which comprises human DCR5 fused to an immunoglobulin constant region, wherein the immunoglobulin constant region is the Fc portion of human IgG1.

In a preferred embodiment, a ligandbody may be used in a method of
5 treatment of the human or animal body, or in a method of diagnosis.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides DCR5 proteins which include natural DCR5
10 proteins and recombinant proteins comprising a DCR5 amino acid sequence, or a functional DCR5 protein domain thereof having an assay-discernable DCR5-specific activity. Accordingly, the proteins may be deletion mutants of the disclosed natural DCR5 proteins and may be provided as fusion products, e.g., with non-DCR5 polypeptides. The subject DCR5 protein domains have DCR5-
15 specific activity or function and are functionally distinct from each other and from DAN, cerberus, Gremlin and Noggin homologs. Such domains include at least 6 and preferably at least 8 consecutive residues of a natural DCR5 protein (See DAN sequence reported by Enomoto, et al. (1994) Oncogene 9: 2785-2791). Preferred DCR5 proteins comprise a DCR5 sequence conserved across species.

20 The DCR5 proteins described herein are structurally and functionally related to DAN and Cerberus in that they are extracellularly active as antagonists of certain morphogenetic proteins such as BMPs. DCR5-specific activity or function may be determined by convenient in vitro, cell-based, or in vivo assays - e.g., in vitro binding assays, cell culture assays, in animals (e.g., immune
25 response, gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the specific molecular interaction of a DCR5 protein with a binding target is evaluated. The binding target may be a natural binding target such as a TGF β protein, a morphogenetic protein, preferably a bone morphogenetic protein such as BMP2 or BMP4, a chaperon, or other regulator that directly modulates DCR5
30 activity or its localization; or non-natural binding target such as a specific immune protein such as an antibody, or a DCR5 specific agent such as those identified in assays described below. Generally, binding specificity is assayed by

bioassay (e.g., the ability to induce neuronal tissue from injected embryonic ectoderm), TGF β protein binding equilibrium constants (usually at least about 10^7 M⁻¹, preferably at least about 10^8 M⁻¹, more preferably at least about 10^9 M⁻¹), by the ability of the subject protein to function as negative mutants in DCR5-
5 expressing cells, to elicit DCR5 specific antibody in a heterologous host (e.g., a rodent or rabbit), etc.

The claimed proteins may be isolated or pure - an "isolated" protein is one that is no longer accompanied by some of the material with which it is associated in its natural state, and that preferably constitutes at least about 0.5%, and more
10 preferably at least about 5% by weight of the total protein in a given sample; a "pure" protein constitutes at least about 90%, and preferably at least about 99% by weight of the total protein in a given sample. The subject proteins and protein domains may be synthesized, produced by recombinant technology, or purified from cells. A wide variety of molecular and biochemical methods are available
15 for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

The subject proteins find a wide variety of uses including use as
20 immunogens, targets in screening assays, bioactive reagents for modulating cell growth, differentiation and/or function, etc. For example, the invention provides methods for modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium surrounding the cell with an exogenous DCR5 protein under conditions whereby the added protein
25 specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. According to these methods, the extracellular surface includes plasma membrane-associated receptors; the exogenous DCR5 refers to a protein not made by the cell or, if so, expressed at non-natural levels, times or physiologic locales; and suitable media
30 include in vitro culture media and physiological fluids such as blood, synovial fluid, etc. Effective administrations of subject proteins can be used to reduce undesirable (e.g., ectopic) bone formation, inhibit the growth of cells that require

a morphogenetic protein (e.g., BMP-dependent neuroblastomas and gliomas), alter morphogen-dependent cell fate/differentiation in culture, such as with cells for transplantation or infusion, etc. The proteins may be introduced, expressed, or repressed in specific populations of cells by any convenient way such as
5 microinjection, promoter-specific expression of recombinant enzyme, targeted delivery of lipid vesicles, etc.

The invention provides natural and non-natural DCR5-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. DCR5-specific binding
10 agents include DCR5-specific ligands such as BMPs, and receptors, such as somatically recombined protein receptors like specific antibodies or T-cell antigen receptors (See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and also includes other natural binding agents identified with assays such as one-, two- and three-hybrid screens, and non-
15 natural binding agents identified in screens of chemical libraries such as described below. Agents of particular interest modulate DCR5 function.

The invention provides DCR5 nucleic acids, which find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc., as well as use in detecting the presence of
20 DCR5 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional DCR5 homologs and structural analogs.

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e., no longer accompanied by some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, more
25 preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Nucleic acids comprising the nucleotide sequence of SEQ ID NO. 11 or fragments thereof, contain such sequence or
30 fragment at a terminus, immediately flanked by a sequence other than that to which it is joined on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which is immediately flanked

by a sequence other than that to which it is joined on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

5 The amino acid sequences of the disclosed DCR5 proteins are used to back translate DCR5 protein-encoding nucleic acids optimized for selected expression systems (Holler, et al. (1993) Gene 136: 323-328; Martin, et al. (1995) Gene 154: 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural DCR5 encoding nucleic acid sequences ("GCG" software,
10 Genetics Computer Group, Inc., Madison, WI). DCR5 encoding nucleic acids may be part of expression vectors and may be incorporated into recombinant host cells, e.g., for expression and screening, for transgenic animals, for functional studies such as the efficacy of candidate drugs for disease associated with DCR5 mediated signal transduction, etc. Expression systems are selected and/or
15 tailored to effect DCR5 protein structural and functional variants through alternative post-translational processing.

 The invention also provides for nucleic acid hybridization probes and replication/amplification primers having a DCR5 cDNA specific sequence and sufficient to effect specific hybridization with SEQ ID NO. 11. Demonstrating
20 specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO₄, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C
25 and remaining bound when subject to washing at 42°C with 0.2x SSPE buffer at 42°C. DCR5 cDNA homologs can also be distinguished from other protein using alignment algorithms, such as BLASTX (Altschul, et al. (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215: 403-410).

 DCR5 hybridization probes find use in identifying wild-type and mutant
30 alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical

diagnoses. DCR5 nucleic acids are also used to modulate cellular expression or intracellular concentration or availability of active DCR5. DCR5 inhibitory nucleic acids are typically antisense - single stranded sequences comprising complements of the disclosed natural DCR5 coding sequences. Antisense

5 modulation of the expression of a given DCR5 protein may employ antisense nucleic acids operably linked to gene regulatory sequences. Cells are transfected with a vector comprising a DCR5 sequence with a promoter sequence oriented such that transcription of the gene yields an antisense transcript capable of binding to endogenous DCR5 encoding mRNA. Transcription of the antisense

10 nucleic acid may be constitutive or inducible and the vector may provide for stable extrachromosomal maintenance or integration. Alternatively, single-stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given DCR5 protein may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results in a substantial reduction in

15 expression of the targeted protein. An enhancement in DCR5 expression is effected by introducing into the targeted cell type DCR5 nucleic acids which increase the functional expression of the corresponding gene products. Such nucleic acids may be DCR5 expression vectors, vectors which upregulate the functional expression of an endogenous allele, or replacement vectors for

20 targeted correction of mutant alleles. Techniques for introducing the nucleic acids into viable cells are known in the art and include retroviral-based transfection, viral coat protein-liposome mediated transfection, etc.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of DCR5

25 modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate DCR5 interaction with a natural DCR5 binding target. A wide variety of assays for binding agents are provided including protein-protein binding assays, immunoassays, cell based assays, etc. Preferred methods are amenable to automated, cost-effective high throughput

30 screening of chemical libraries for lead compounds.

In vitro binding assays employ a mixture of components including a DCR5 protein, which may be part of a fusion product with another peptide or

polypeptide, e.g., a tag for detection or anchoring, etc. The assay mixtures comprise a natural DCR5 binding target, e.g., a TGF β protein such as a BMP. While native binding targets may be used, it is frequently preferred to use portions thereof as long as the portion provides binding affinity and avidity to the subject DCR5 conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds, preferably small organic compounds, and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents such as salts, buffers, neutral proteins, e.g., albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., may also be included. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. The mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the DCR5 specifically binds the cellular binding target, portion or analog with a reference binding affinity. Incubation periods are chosen for optimal binding but are also minimized to facilitate rapid, high throughput screening.

After incubation, the agent-biased binding between the DCR5 and one or more binding targets is detected by any convenient way. For cell-free binding type assays, a separation step is often used to separate bound from unbound components. Separation may be effected by precipitation, immobilization, etc., followed by washing by, e.g., membrane filtration or gel chromatography. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc., or indirect detection such as an epitope tag, an enzyme, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g., through optical or electron density, radiative emissions, nonradiative energy transfers, or indirectly detected with antibody conjugates, etc. A difference in the binding affinity of the DCR5 protein to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates

the binding of the DCR5 protein to the corresponding binding target. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

5 The invention provides for a method for modifying the physiology of a cell comprising an extracellular surface in contact with a medium, said method comprising the step of contacting said medium with an exogenous DCR5 protein under conditions whereby said protein specifically interacts with at least one of a component of said medium and said extracellular surface to effect a change in the physiology of said cell.

10 The invention further provides for a method for screening for biologically active agents, said method comprising the steps of a) incubating a DCR5 protein in the presence of an extracellular DCR5 protein specific binding target and a candidate agent, under conditions whereby, but for the presence of said agent, said protein specifically binds said binding target at a reference affinity; b)
15 detecting the binding affinity of said protein to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said protein to said binding target.

The invention also provides for the production of ligandbodies.

20 Ligandbodies are comprised of a ligand polypeptide coupled to the Fc domain of IgG and are able to dimerize (see for example Davis, et al., 1994, Science 266:816-819). Ligandbodies have the advantage of exhibiting enhanced pharmacokinetic properties. Thus, DCR5 ligandbodies may be useful in therapeutic applications where enhanced pharmacokinetic properties of DCR5 is desirable.

25 One embodiment of the invention is an isolated DCR5 protein comprising the amino acid sequence as set forth in SEQ ID NO. 12 or a fragment thereof having DCR5- specific activity.

Another embodiment of the invention is a recombinant nucleic acid encoding DCR5 protein comprising the amino acid sequence as set forth in SEQ
30 ID NO. 12 or a fragment thereof having DCR5- specific activity.

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth in SEQ ID NO. 11 or a fragment thereof having at

least 18 consecutive bases of SEQ ID NO. 11 and sufficient to specifically hybridize with a nucleic acid having the sequence of SEQ ID NO. 11 in the presence of natural DAN and cerberus cDNA.

Another preferred embodiment of the invention is a method of treatment
5 of a human or animal body by administering a therapeutic dosage of a human DCR5 polypeptide as wherein the treatment is regulation of cartilage and bone growth.

The present invention also provides for antibodies to the DCR5 protein described herein which are useful for detection of the protein in, for example,
10 diagnostic applications. For preparation of monoclonal antibodies directed toward this DCR5 protein, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature* 256:495-497), as well as the trioma technique, the human B-cell
15 hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies for diagnostic or therapeutic use may be
20 human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:7308-7312; Kozbor et al., 1983, *Immunology Today* 4:72-79; Olsson et al., 1982, *Meth. Enzymol.* 92:3-16). Chimeric antibody molecules may be prepared
25 containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81:6851, Takeda et al., 1985, *Nature* 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of the DCR5 protein described herein. For the
30 production of antibody, various host animals can be immunized by injection with the DCR5 protein, or a fragment or derivative thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the

immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, polypeptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected DCR5 protein epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

The invention further provides for a method of using a DCR5 protein or fragment thereof as an antagonist of the activity of a bone morphogenetic protein (BMP), either alone, or in combination with other factors, including DAN, Cerberus, b57 or von Willebrand factor to regulate or modulate the activity of a BMP.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Cloning of human DCR5

5

Searching of the nucleotide database at NCBI using the human Gremlin (hGRE) nucleotide sequence as a query, pooled out an entry encoding for a murine protein termed PRDC [Minabe-Saegusa, 1998], for "Protein Related to DAN and Cerberus". PCR was used to clone the murine PRDC from genomic DNA as well as several murine cDNA sources (brain, smooth muscle, skeletal muscle, liver, embryonic day 15 embryo, embryonic day 17 embryo). The open reading frame (ORF) encoding murine PRDC lies on a single exon. We extrapolated that if the PRDC ORF lies on a single exon, then its human homolog, which was designated human DCR5 (for human DAN/Cerberus related gene number 5), should also lie on a single exon, allowing the use of human genomic DNA in screening for the complete ORF of this gene. Furthermore, we used the PRDC ORF as a probe to investigate the expression of the putative human DCR5 on Multiple Human Tissue Northern blots (Clontech), and it appeared to be expressed in brain, placenta, liver, skeletal muscle, small intestine, colon (mucosal lining), stomach, colon (muscle) , small intestine (muscle), bladder (muscle), stomach (muscle), and prostate (muscle), thus pointing to several sources of cDNA that could be used to clone the hDCR5 cDNA.

The peptide sequence of PRDC was aligned to all the known members of the DAN/Cerberus (DAN/CER) family and regions that are highly conserved were determined. One of the most striking features of the proteins belonging to the DNA/CER family is a conserved pattern of cysteines (CYS). Four different amino acid sequences that contained some of these Cys residues were chosen to design four degenerate oligonucleotide primers with the following sequences:

30

10 20
* *

MGN AAR TAY YTN AAR WSN GAY TGG TGY
5 (Arg) Lys Tyr (Leu) Lys (Ser) Asp Trp Cys>
65 73

			10			20	
10		*				*	
	CAR ACN GTN WSN GAR GAR GGN TGY						
	Gln Thr Val (Ser) Glu Glu Gly Cys>						
	80						87

	10	20
	*	*
	NGG NGG RTC NAR NCC NGG RCA	
	<Pro Pro Asp (Leu) Gly Pro Cys	
20	143	137

	10	20
	*	*
25	NAR RTT NAC NSW CAT RCA NCK RCA	
	<(Leu) Asn Val (Ser) Met Cys (Arg) Cys	
	162	155

30 Degenerate bases are indicated using IUPAC nomenclature.
Amino acids in parentheses indicate the amino acid present at that position in murine PRDC.

Numbers in *italics* indicate the amino acid number in the sequence of PRDC protein (starting with the initiating Methionine as amino acid number 1).

The direction of the amino acid sequence is reversed for primers DCR5.d3-3' (SEQ ID NO: 3) and DCR5.d4-3' (SEQ ID NO: 4) as indicated by arrows (<).

5

We employed primers DCR5.d1-5' (SEQ ID NO: 1) and DCR5.d4-3' (SEQ ID NO: 4) (i.e. the outermost primers in the sequence of PRDC) to set up PCR using human genomic DNA as the template, using standard PCR methodology. The products of these reaction were used as a template for a second set of PCR

10 reactions using primers DCR5.d2-5' (SEQ ID NO: 2) and DCR5.d3-3' (SEQ ID NO: 3). This second PCR reactions amplified a DNA fragment of approximately 200 base pairs, which was close to the size expected according to the PRDC sequence. This fragment was subcloned into the plasmid vector pUC18 using standard genetic engineering methodology, and then sequenced. This sequence showed
15 very high homology to PRDC, indicating that this was indeed a fragment of human DCR5. Based on this information, exact primers corresponding to the 5' end and the 3' end of this sequence were engineered as shown below:

A. Sequence of fragment of human DCR5 generated by PCR:

```

20          10          20          30          40
          *          *          *          *
SEQ ID NO:5 CAG ACA GTG ACG GAG GAG GGC TGC CGG AGC CGC ACC ATC CTC AAC CGC
          GTC TGT CAC TGC CTC CTC CCG ACG GCC TCG GCG TGG TAG GAG TTG GCG
SEQ ID NO:6 Gln Thr Val Thr Glu Glu Gly Cys Arg Ser Arg Thr Ile Leu Asn Arg>
25
          50          60          70          80          90
          *          *          *          *          *
          TTC TGC TAC GGC CAG TGC AAC TCC TTC TAC ATC CCG CGG CAC GTG AAG
          AAG ACG ATG CCG GTC ACG TTG AGG AAG ATG TAG GGC GCC GTG CAC TTC
30 Phe Cys Tyr Gly Gln Cys Asn Ser Phe Tyr Ile Pro Arg His Val Lys>

          100          110          120          130          140
          *          *          *          *          *
          AAG GAG GAG GAG TCC TTC CAG TCC TGC GCC TTC TGC AAG CCC CAG CGC

```

TTC CTC CTC CTC AGG AAG GTC AGG ACG CGG AAG ACG TTC GGG GTC GCG
Lys Glu Glu Glu Ser Phe Gln Ser Cys Ala Phe Cys Lys Pro Gln Arg>

5 150 160 170 180 190
 * * * * *
GTC ACC TCC GTC CTC GTG GAG CTC GAG TGC CCG GGA CTA GAC CCC CCA
CAG TGG AGG CAG GAG CAC CTC GAG CTC ACG GGC CCT GAT CTG GGG GGT
Val Thr Ser Val Leu Val Glu Leu Glu Cys Pro Gly Leu Asp Pro Pro>

10 **Key:**

The primers h/mDCR5.in1-5' and hDCR5.in2rev are underlined.

B. Sequence of primers synthesized based on the human DCR5 partial genomic sequence:

15 (i) primer h/mDCR5.in1-5':

 10 20 30
 * * *
SEQ ID NO: 7 AGC CGC ACC ATC CTC AAC CGC TTC TGC TAC
SEQ ID NO: 8 Ser Arg Thr Ile Leu Asn Arg Phe Cys Tyr>

20

(ii) primer hDCR5.in2rev:

 10 20
 * *
SEQ ID NO: 9 CTC GAG CTC CAC GAG GAC GGA GGT GAC
25 SEQ ID NO: 10 <Glu Leu Glu Val Leu Val Ser Thr Val

These primers were used in PCR to amplify a 140 bp fragment using liver cDNA as a template. To clone the full length hDCR5 ORF, we screened a Rapid Screen Human Liver cDNA Library Panel (OriGene Technologies, Inc.) by PCR
30 using the above primers. This lead to the identification of several independent cDNA clones. Sequencing of one of these clones revealed the existence of a 507 bp ORF that encodes for a 168 amino acid polypeptide which we have designated human DCR5. The sequence, which is set forth below, has very high sequence identity (95%) to PRDC. In addition, it bears 65% sequence identity with human
35 Gremlin, and 28 and 26% sequence identity with Cerberus and DAN,

respectively, all of which are members of a family which comprise a conserved cysteine pattern and consensus sequence and all of which function as BMP antagonists.

The nucleotide sequence (SEQ ID NO: 11) and amino acid sequence (SEQ ID NO: 12) of the human DCR5 open reading frame was determined to be as follows:

```

      10      20      30      40      50
      *      *      *      *      *      *      *      *      *      *
10  ATG TTC TGG AAG CTT TCC CTG TCC TTG TTC CTG GTG GCG GTG CTG GTG AAG GTG GCG
    Met Phe Trp Lys Leu Ser Leu Ser Leu Phe Leu Val Ala Val Leu Val Lys Val Ala>

      60      70      80      90      100      110
      *      *      *      *      *      *      *      *      *      *
15  GAA GCC CGG AAG AAC CGG CCG GCG GCC ATC CCC TCG CCT TAC AAG GAC GGC AGC
    Glu Ala Arg Lys Asn Arg Pro Ala Gly Ala Ile Pro Ser Pro Tyr Lys Asp Gly Ser>

      120      130      140      150      160      170
      *      *      *      *      *      *      *      *      *      *
20  AGC AAC AAC TCG GAG AGA TGG CAG CAC CAG ATC AAG GAG GTG CTG GCC TCC AGC CAG
    Ser Asn Asn Ser Glu Arg Trp Gln His Gln Ile Lys Glu Val Leu Ala Ser Ser Gln>

      180      190      200      210      220
      *      *      *      *      *      *      *      *      *      *
25  GAG GCC CTG GTG GTC ACC GAG CGC AAG TAC CTC AAG AGT GAC TGG TGC AAG ACG CAG
    Glu Ala Leu Val Val Thr Glu Arg Lys Tyr Leu Lys Ser Asp Trp Cys Lys Thr Gln>

      230      240      250      260      270      280
      *      *      *      *      *      *      *      *      *      *
30  CCG CTG CGG CAG ACG GTG AGC GAG GAG GCC TGC CGG AGC CGC ACC ATC CTC AAC CGC
    Pro Leu Arg Gln Thr Val Ser Glu Glu Gly Cys Arg Ser Arg Thr Ile Leu Asn Arg>

      290      300      310      320      330      340
      *      *      *      *      *      *      *      *      *      *
35  TTC TGC TAC GGC CAG TGC AAC TCC TTC TAC ATC CCG CGG CAC GTG AAG AAG GAG GAG
    Phe Cys Tyr Gly Gln Cys Asn Ser Phe Tyr Ile Pro Arg His Val Lys Lys Glu Glu>

```

```

      350      360      370      380      390
      *      *      *      *      *      *      *      *      *
GAG TCC TTC CAG TCC TGC GCC TTC TGC AAG CCC CAG CGC GTC ACC TCC GTC CTC GTG
Glu Ser Phe Gln Ser Cys Ala Phe Cys Lys Pro Gln Arg Val Thr Ser Val Leu Val>

      400      410      420      430      440      450
      *      *      *      *      *      *      *      *      *
GAG CTC GAG TGC CCC GGC CTG GAC CCA CCC TTC CGA CTC AAG AAA ATC CAG AAG GTG
Glu Leu Glu Cys Pro Gly Leu Asp Pro Pro Phe Arg Leu Lys Lys Ile Gln Lys Val>

      460      470      480      490      500
      *      *      *      *      *      *      *      *      *
AAG CAG TGC CGG TGC ATG TCC GTG AAC CTG AGC GAC TCG GAC AAG CAG TGA
Lys Gln Cys Arg Cys Met Ser Val Asn Leu Ser Asp Ser Asp Lys Gln ***>

```

The sequence of the human DCR5 polypeptide (SEQ ID NO: 12) was determined to be as follows:

```

                                >N_Glycos
                                |
      10      20      30      40      50
      *      *      *      *      *
MFWKLSLSLFLVAVLVKVAEARKNRPAGAIIPSPYKDGSSNNSERWQHQIK

      60      70      80      90      100
      *      * ‡      *      ‡ *      ‡ *
EVLASSQEALVVTERKYLKSDWCKTQPLRQTVSEEGCRSRTILNRF CYGQ

      110      120      130      140      150
      ‡      *      ‡ ‡      *      ‡ *      *
CNSFYIPRHVKKEEESFQSCAFCKPQRVTSVLVELECPGLDPPFRLKKIQ

      >N_Glycos
      |
      160 |
      ‡ ‡ * |
KVKQCRCMSVNLSDSDKQ*

```

Key:

The signal peptide region is underlined.

>N_Glycos marks the position of the predicted N-linked glycosylation sites.

‡ marks the position of the cysteines that are conserved among the members of
5 the DAN/Cerberus family.

The human DCR5 DNA sequence set forth in SEQ ID NO. 11 *supra* is in
the cloning vector pCMV6-XL3 (OriGene Technologies, Inc.), and is designated
pCAE626.

10

Example 2Construction and expression of human DCR5-myc3

15 The human DCR5 open reading frame (ORF) was amplified by PCR using
two primers:

SEQ ID NO. 13:

20 hDCR5 PCR5' (Eco) (5'-CAG ATA GAA TTC GCC GCC ACC ATG GTG TGG AAG
CTT TCC CTG TCC TTG-3')

SEQ ID NO. 14:

25 hDCR5 PCR3' (AgeI) (5'-CAC GAG ACC GGT CTG CTT GTC CGA GTC GCT-3')

PCR amplification was performed using ExTaq DNA Polymerase
(TaKaRa). The PCR product was purified away from excess primers, digested
with the restriction endonucleases EcoRI and AgeI, gel-purified and subcloned
30 into the mammalian expression vector pMT21-myc3 at the EcoRI and AgeI
cloning sites, bringing the human DCR5 ORF (minus a stop codon) in frame with
a triple myc-tag, the sequence of which is set forth below:

SEQ ID NO. 15:

5'-GAG CAG AAG CTG ATA TCC GAA GAA GAC CTC GGC GGA GAG CAG
AAG CTC ATA AGT GAG GAA GAC TTG GGC GGA GAG CAG AAG CTT ATA
5 TCC GAA GAA GAT CTC GGA CCG TGA TAA-3'

This triple myc tag is contained in the vector immediately 3' to the unique AgeI site that was used for cloning purposes. The final DNA construct was verified by standard restriction analysis and dideoxy sequencing.

10

Human DCR5-myc3 protein was expressed in COS7 cells that were transiently transfected with the pMT21/human DCR5-myc3 DNA construct described *supra*. The transfection was done using Lipofectamine (Life Technologies, Inc.) as described by the manufacturer. Serum-free conditioned
15 media were collected two days after transfection and cleared of cell debris by low speed centrifugation. EDTA was added to the conditioned media to a concentration of 5mM. The conditioned media were aliquoted and stored at -20°C. The expression of human DCR5-myc3 was verified by standard western blotting techniques using an anti-myc monoclonal antibody (9E10; 1 µg/ml)
20 against the myc tag. Under standard non-reducing conditions, human DCR5-myc3 displayed an approximate molecular size of 30,000, which is consistent with the predicted molecular size of human DCR5-myc3 when accounting for glycosylation at the two potential N-linked glycosylation sites.

25

Example 3

Human DCR5 binds to BMP2 and BMP4 but not other BMPs

Human DCR5-myc3 (1 ml of COS7 cell-derived serum-free conditioned media described *supra*) was co-incubated with human BMP-2 (1 µg/ml), or
30 human BMP-4 (0.5 µg/ml) (R&D Systems), or human BMP-5 (1 µg/ml) (R&D Systems), or mouse Nodal (also known as mBMP-16; provided as ³⁵S-mouse Nodal expressed in *X. laevis* oocytes), or human BMP-11 (also known as human

GDF-11; provided as ^{35}S -human BMP-11 expressed in *X. laevis* oocytes), in the absence or in the presence of human noggin protein hNGAB2 (described in published PCT application publication no. WO 99/03996, published January 28, 1999, and incorporated in its entirety herein by reference), 2 to 5 $\mu\text{g}/\text{ml}$. The
5 formation of a stable complex between human DCR5-myc3 and the different BMP family members was determined by immunoprecipitating human DCR5-myc3 and associated proteins using the anti-myc monoclonal antibody (9E10; 1 $\mu\text{g}/\text{ml}$) bound to Protein A Ultralink (Pierce). The binding reaction was carried out in the serum-free conditioned media containing 20 mM Tris pH 7.6, 150 mM
10 NaCl, 0.1% Tween 20 (TBST), and 1 mg/ml bovine serum albumin (BSA). Binding was allowed to proceed for 1 hour at 25°C in a reaction volume of 1.1 ml, with continuous mixing to keep the Protein A-Ultralink (Pierce) in suspension. Following incubation, the beads were pelleted by low speed centrifugation, washed once with TBST, transferred to fresh eppendorf tubes, and washed 3
15 additional times with TBST. Protein bound to the beads was solubilized by the addition of 25 μl of standard Laemli SDS-PAGE sample buffer and loaded onto 4 to 12% NuPAGE/MES gradient gels (Novex), which were run under standard reducing conditions. The electrophoresed proteins were subsequently transferred onto Immobilon P membranes and probed for the presence of human BMP-2 or
20 human BMP-4 or human BMP-5 using antisera raised against each respective protein.

In one example, human DCR5-myc3 was shown to bind to human BMP-2. This interaction was blocked by the inclusion of 2 μg of hNGAB2 in the binding reaction. This result indicates that the interaction between human BMP-2 and
25 human DCR5-myc3 is specific. There was no binding of human BMP-2 to the beads when human DCR5-myc3 was not included in the binding reaction either in the presence or absence of hNGAB2.

In another example, the ability of human DCR5-myc3 to bind to human BMP-4 was tested. Human DCR5-myc3 was able to bind human BMP-4. In
30 addition, the interaction of human BMP-4 with human DCR5-myc3 was blocked by addition of hNGAB2 (5 μg). As described above for human BMP-2, there was no binding of human BMP-4 to the beads when human DCR5-myc3 was omitted

from the reaction mixture either in the presence or absence of hNGAB2. Human DCR5 expressed in *E. Coli*, purified and refolded (see *infra*) was tested for its ability to compete with human DCR5-myc3 for binding to hBMP4. Inclusion of 5 µg of *E. coli*-expressed, purified and refolded human DCR-5 was able to block binding of human DCR5-myc3 to hBMP-4, indicating that this refolded human DCR-5 is active.

The ability of human DCR-5 to bind to other members of the BMP family was also tested. The results are as follows: hDCR-5 does not bind human BMP-5, mouse Nodal (mBMP-16), or human BMP-11 (also known as GDF-11). The lack of interaction with these BMPs provides further evidence that human DCR-5 is a specific antagonist of BMP-2 and BMP-4. However, the possibility that human DCR-5 binds to and blocks the activity of other BMP family members cannot be excluded.

15

Example 4

Construction of human DCR5 *E. Coli* expression plasmid pRG764

A DNA fragment encoding the gene for human DCR5 was PCR amplified by standard techniques using the plasmid pCAE626 (described *supra*) as a template and the following oligonucleotides as amplification primers:

20
SEQ ID NO. 16:25
N1-hDCR5 (5'-GAGAGACATGTCT CGGAAGAACCGTCCGGCTGGCGCC
ATCCCCTCGCCTTAC-3')

SEQ ID NO. 17:

30
C1-hDCR5 (5'-GAGAGCGGCCGCTCATTACTGCTTGTCCGAGTCGCT
CAG-3').

The resulting 472 bp fragment includes nucleotides 64-504 of SEQ ID NO. 11 plus additional sequence for cloning (underlined in the oligonucleotide primer sequences *supra*) and encodes the mature human DCR5 gene starting with arginine at position 22 of SEQ ID NO. 12, which was determined by computer analysis to be the first amino acid after the signal sequence cleavage site. To facilitate cloning in *E. coli*, the human DCR5 encoding sequence was preceded by the codons for methionine and serine which introduced an Afl III restriction site for cloning. The codons for arginine and alanine at positions 25 and 27 in the human DCR5 sequence were changed from CGG and GCG to CGT and GCT, respectively, to reduce the GC content of the sequence proximal to the translation initiation site. These changes are silent mutations and do not alter the amino acid sequence. The resulting DNA fragment was digested with the restriction endonucleases Afl III, then ligated using standard techniques into the NcoI and EagI cloning sites in the *E. coli* expression plasmid pRG663. The resulting plasmid, designated pRG764, contains the human DCR5 gene under transcriptional control of the T7 F1.1 promoter in a high copy number plasmid encoding the kanamycin resistance gene. The plasmid was confirmed by restriction enzyme analysis and DNA sequence determination using standard techniques known to skilled artisans.

20

Example 5

E. Coli expression, purification and refolding of human DCR5 protein.

Plasmid pRG764 (described *supra*) was transformed into the prototrophic *E. coli* K12 expression strain RFJ143 using standard transformation techniques. Strain RFJ143 expresses the phage T7 RNA polymerase under transcriptional control of the *lacUV5* promoter. *E. coli* strain RFJ143 containing the pRG764 plasmid was grown in LB medium + 20µg/ml kanamycin. Expression of human DCR5 protein was induced by the addition of 1 mM IPTG. Induced cells were collected by centrifugation at 10,000g for 10 minutes, resuspended in 10 volumes of 100 mM Tris-HCl, pH 8.5, 20 mM EDTA, and lysed by passage through a Niro-Soave Panda cell disrupter. The cell lysate was centrifuged at 10,000g for 10

30

minutes and the pellet was resuspended in 10 volumes of 8 M guanidine-HCl, 50 mM Tris-HCl, pH 8.5, 1 mM EDTA, 100 mM Na₂SO₃, 10 mM Na₂S₄O₆ and stirred for 16 hours at room temperature. The solubilized inclusion bodies were fractionated on a Sephacryl S-300 column (Pharmacia) equilibrated in 8 M urea, 50 mM MES, pH 6.0, 200 mM NaCl, 1 mM EDTA. Fractions containing human DCR5 were pooled and diluted with 4 volumes of buffer containing 6 M urea, 20 mM MES, pH 6.0. The diluted human DCR5 pool was loaded onto an SP-Sepharose ion exchange column (Pharmacia) equilibrated with 6 M urea, 20 mM MES, pH 6.0 then eluted by a linear gradient ranging from 0 to 1 M NaCl in 6 M urea, 20 mM MES, pH 6.0. Fractions containing purified human DCR5 were pooled then diluted to about 0.1 mg/ml human DCR5 with 50 mM Tris-HCl, pH 8.5. Cysteine was added to 0.5 mM and NaCl was added to 1 M. This refold mix was incubated at 4°C for 5 days with gentle stirring. Refolded human DCR5 was collected and purified by reversed-phase chromatography on a Phenomenex Jupiter C5 column run with a linear gradient from 0.1% TFA in H₂O to 0.1% TFA in acetonitrile. Fractions containing refolded human DCR5 were pooled, dried under vacuum, and resuspended in PBS.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding human DCR5.
2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
 - (a) the nucleotide sequence comprising the coding region of the human DCR5 as set forth in SEQ ID NO. 11;
 - (b) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the human DCR5; or
 - (c) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleic acid of (a) or (b) and which encodes human DCR5.
3. A vector which comprises a nucleic acid molecule of claim 1 or 2.
4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.
5. A vector according to claim 3 or 4, which is a plasmid.
6. Isolated human DCR5 protein.
7. Isolated human DCR5 protein, having the amino acid sequence as set forth in SEQ ID NO. 12.
8. A host-vector system for the production of human DCR5 which comprises a vector of claim 3, 4, or 5 in a host cell.

9. A host-vector system according to claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.
10. A method of producing human DCR5 which comprises growing cells of a host-vector system of claim 8 or 9, under conditions permitting production of the human DCR5, and recovering the human DCR5 so produced.
11. An antibody which specifically binds the human DCR5 of claim 6 or 7.
12. An antibody according to claim 11, which is a monoclonal antibody.
13. A composition comprising human DCR5 according to claim 6 or 7, and a carrier.
14. A composition comprising an antibody according to claim 11 or 12, and a carrier.
15. Human DCR5 according to claim 6 or 7, an antibody according to claim 11 or 12, or a composition according to claim 13 or 14, for use in a method of treatment of the human or animal body, or in a method of diagnosis.
16. A polypeptide produced by the method of claim 10.
17. A ligandbody which comprises human DCR5 fused to an immunoglobulin constant region.
18. The ligandbody of claim 17, wherein the immunoglobulin constant region is the Fc portion of human IgG1.

19. A ligandbody according to claim 17 or 18, for use in a method of treatment of the human or animal body, or in a method of diagnosis.
20. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9.
21. A method of regulating cartilage and bone growth comprising administering the human DCR5 or antibody or composition of claim 15.

INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/US 99/17979

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/62 C12N5/10 C12N1/21 C12N1/19
C07K14/475 C07K16/22 A61K38/18 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MINABE-SAEGUSA C ET AL: "Sequence and expression of a novel mouse gene PRDC (protein related to DAN and cerberus) identified by a gene trap approach" DEV GROWTH DIFFER, vol. 40, no. 3, June 1998 (1998-06), pages 343-353, XP000870237 cited in the application figure 5	1-16,20
A	WO 98 33918 A (UNIV CALIFORNIA) 6 August 1998 (1998-08-06)	21

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 January 2000

Date of mailing of the international search report

03/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lonnoy, 0

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/17979

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HSU D R ET AL: "The Xenopus dorsalizing factor Gremlin identifies a novel family of secreted proteins that antagonize BMP activities"</p> <p>MOLECULAR CELL,US,CELL PRESS, CAMBRIDGE, MA,</p> <p>vol. 1, no. 5, page 673-683-683</p> <p>XP002113640</p> <p>ISSN: 1097-2765</p> <p>---</p>	21
P,A	<p>WO 99 06552 A (LACROIX BRUNO ;DUCLERT AYMERIC (FR); GENSET (FR); DUMAS MILNE EDWA) 11 February 1999 (1999-02-11)</p> <p>SeqIdNo.55: 98.7% identity in 78 bp overlap with SeqIdNo.11 / SeqIdNo.288: 100.0% identity in 25 aa overlap in first 25aa of SeqIdNo.12</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/17979

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 21 as far as an in vivo is concerned,
is directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/17979

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9833918	A	06-08-1998	AU 6267098 A	25-08-1998
WO 9906552	A	11-02-1999	AU 8555598 A	22-02-1999